Ultrarapid, Convection-Enhanced Intravascular Hypothermia
A Feasibility Study in Nonhuman Primate Stroke

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Background and Purpose—Hypothermia has been shown to be neuroprotective in a variety of clinical settings. Unfortunately, poor delivery techniques and insufficient data in appropriate preclinical models have hampered its development in human stroke. To address these limitations, we have devised a 10F intravascular catheter capable of rapid systemic cooling of nonhuman primates.

Methods—Placed in the inferior vena cava via a transfemoral approach, the catheter was used to induce mild systemic hypothermia 3 hours after the onset of hemispheric stroke in baboons.

Results—Cooling was achieved at a rate of $6.3\pm0.8^\circ C/h$. Target brain temperatures ($32.2\pm0.2^\circ C$) were reached at the same time (47.7$\pm$6.32 minutes) as target esophageal temperatures ($32.0\pm0.0^\circ C$). Hypothermia was maintained for 6 hours in all animals. Animals did not experience the infections, coagulopathy, or cerebral edema commonly seen with surface cooling methods in human stroke.

Conclusions—These data suggest that a brief episode of mild core hypothermia instituted at a clinically relevant time point can be achieved in primate stroke and that our intravascular cooling technique provides safe, rapid, and reproducible hypothermia. (Stroke. 2003;34:1994-1999.)

Key Words: hypothermia ■ primates ■ stroke ■ baboons
achieve reproducible total body cooling in a nonhuman primate model of stroke.

Methods

Animals
Sixteen male baboons (Papio anubis; Buckshire Farms; 21.1±0.9 kg) were included in this series after an appropriate period of quarantine and observation. All animal care and experimental procedures were approved by the Institutional Animal Care and Use Committee and performed in accordance with the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals.

Anesthesia
Animals were subjected to anesthesia and monitoring as previously described. Baboons were anesthetized with 5 mg/kg IM ketamine (Fort Dodge Animal Health). Two 18-gauge peripheral venous catheters were placed, and intravenous fluid administration of 0.9% normal saline was begun. Propofol (Zeneca Pharmaceutical) was given as a bolus infusion before oropharyngeal intubation. Animals were transferred to an operating room and were begun on assisted ventilation (Ohmeda 7000 ventilator) with an inhalational mixture of isoflurane (Baxter) and balanced nitrous oxide and oxygen. Animals were securely placed into a head frame (Stoelting) in the prone position. In anticipation of placing additional monitoring devices, an intravenous bolus infusion of fentanyl (Elkin-Sims) at 50 g/kg was given, followed by a continuous infusion of 50 to 70 μg·kg⁻¹·h⁻¹. The concentration of isoflurane was maintained between 0% and 0.5%. Intravenous cefazolin (Bristol/Myers Squibb) was administered for antibiotic prophylaxis. Before final positioning, a continuous intravenous vecuronium infusion (Organon) was started at 0.04 mg·kg⁻¹·h⁻¹. At the initiation of the transorbital approach, the rate of fentanyl infusion was increased to 70 to 100 μg·kg⁻¹·h⁻¹, and the isoflurane was decreased to <0.5%.

Physiology
An intra-arterial catheter was introduced into the femoral artery for continuous systemic blood pressure monitoring and multiple blood specimen collections at baseline, during ischemia, and during reperfusion. Mean arterial blood pressure (MABP) and heart rate were monitored. Hypotension was treated with intravenous bolus injections of phenylephrine hydrochloride (Gensia Laboratories) to maintain an MABP of 60 to 80 mm Hg. An indwelling Foley catheter (Baxter) was placed for urinary output monitoring and guidance of fluid management. Arterial blood gas analysis (Pco₂, PH, Po₂) was performed at regular intervals (Stat Profile 3, Nova Biomedical). The respiratory rate and tidal volume were adjusted to keep PCo at 35 to 40 mm Hg. Cerebral parenchymal temperature probes were positioned bilaterally through precoronal burr holes. Core temperature was monitored by placement of an esophageal temperature probe (Mallinckrodt Inc). Continuous intracranial pressure (ICP) monitoring was accomplished with a parenchymal sensor (Neurimonitor, Codman) placed in the right frontal lobe. Sustained ICP of >20 mm Hg for >5 minutes was treated with 0.5 g/kg mannitol as an intravenous bolus infusion. Motor evoked potentials (MEPs) were monitored by applying transcranial electric stimulation to the motor cortex and recording compound muscle action potentials from the forelimbs. Adequacy and uniformity of cerebral ischemia were ascertained by stimulating the ipsilateral ischemic hemisphere and noting contralateral limb MEPs. Preservation of the use of stimuli strong enough to elicit potent contralateral (nonischemic) hemisphere and produce ipsilateral limb MEPs.

Reperfused Stroke
Animals were subjected to middle cerebral artery territory reperfused stroke as previously described by Huang et al. Briefly, they underwent orbital exenteration and removal of the posterior-medial orbital wall under the microscope (Super-Lux 40-2 Illuminator, Zeiss). Dura was opened, and the cerebral arteries were identified.

Hypothermia
Hypothermia was induced in the experimental cohort (n=8) by use of a novel heat-transfer catheter (Innercool Therapies, Inc; Figure 1). This catheter, described previously in a porcine model of intravascular hypothermia, was designed to provide precise, stable, and rapid cooling. The catheter was placed intravenously through the femoral vein into the inferior vena cava. Cooling to a target temperature of 32°C was initiated 3 hours (185.3±17.5 minutes) after the onset of ischemia and continued for a total of 6 hours. Core temperature was used as feedback to control the cooling system, and core and brain temperatures were recorded throughout stroke induction, reperfusion, cooling, and rewarming at 1 Hz (National Instruments). After treatment, the catheter was removed from the vasculature and examined for adherent thrombus. Animals were slowly rewarmed (1.0±0.1°C/h) to normal core temperature (36.5±0.5°C) over 6 hours with surface heating blankets (Mallinckrodt Inc). Control animals (n=8) underwent no cooling and were maintained in the normothermic range (36.7±0.5°C) with heating blankets.

Figure 1. A, Catheter with polymeric shaft and distal metallic heat exchanger. B, Cross section of catheter heat transfer element illustrating closed-loop flow of heat transfer fluid. C, Detail of unique surface features used in the catheter heat transfer element design.
Blood Samples
Blood samples from 4 animals in each group were analyzed for hematocrit, white blood cell count, platelet count, blood glucose, and serum chemistry. Samples were drawn from control animals at baseline, during ischemia, and during reperfusion (12 hours after ischemia). In the hypothermic animals, blood was drawn at baseline, during ischemia, at core temperature of 32°C, during rearming, and at 12 hours after ischemia (normothermia).

Neurological Outcome
Animals were assessed postoperatively on a daily basis with the 100-point Spetzler scale, with higher scores representing better functional outcomes. Motor function was graded from 1 to 75, according to severity of hemiparesis in the extremities (10=severe, 25=mild, 55=favors normal side, and 70=normal) and face (1=fa- normal to 20 (0=dead, 1=comatose, 5=active but inactive, 15=aware but less active, and 20=normal), and visual field deficits were assigned 1 if present or 5 if absent.

Radiographic Imaging
T2-weighted MRI was performed on sedated animals to measure infarct volume at early (72 hours) and late (9 to 10 days) time points, as previously described. Animals were anesthetized with ketamine and sedated with an intravenous pentobarbital bolus and propofol infusion that was titrated to allow independent respiratory function while the airway was maintained with an endotracheal tube. Brain MRI was performed (Signa Advantage, 1.5 T, General Electric) to obtain coronal T2-weighted images with a slice thickness of 3 mm without intervening tissue. T2 characteristics included the following: repetition time, 3000 ms; echo time, 105 ms; excitation, 1; echo train length, 10; field of view, 20×15; and matrix, 256×192. Hypothermic animals also underwent 12-hour gradient-echo and diffusion-weighted imaging to assess for early stroke volume and the presence of hemorrhagic conversion.

Digital planimetric analysis (Adobe Photoshop 4.0 and NIH Image) was performed by 2 independent observers to quantify infarct volume (percent ipsilateral hemisphere).

Euthanasia
Animals incapable of self-caring at 72 hours were euthanized early. All other animals were euthanized late (maximum duration, 10 days). Animals were euthanized with intravenous pentobarbital (Veterinary Laboratories). After euthanasia, animals were perfused intra-arterially with 1 L heparinized saline at 4°C followed by 1 L heparinized paraformaldehyde at 4°C for tissue fixation. Brains were then removed intact and preserved in paraformaldehyde at room temperature.

Statistical Analysis
Ability to self-care was determined for all animals at 72 hours. Infarct volumes and neurological scores were assessed at 72 hours and 9 to 10 days for those capable of survival beyond 72 hours. Values are expressed as mean±SEM. Comparisons between groups were performed with the 2-tailed Student’s t test/nonparametric analysis or χ² test. Statistical significance was defined by P<0.05.

Results

Hypothermia
Cooling was achieved at a rate of 6.3±0.8°C/h (Figure 2). Target brain temperatures (32.2±0.2°C) were reached at the same time (47.7±6.32 minutes) as target esophageal temperatures (32.0±0.0°C). Hypothermia was maintained for 6 hours in all animals. Animals were rewarmed at a rate of 1.0±0.1°C/h until the target core temperature of 36.15±0.2°C was attained. Twelve hours after reperfusion, hypothermic animals underwent gradient-echo MRI, which revealed no hemorrhagic conversion of the infarct. There were no episodes of cardiac arrhythmias during hypothermia or rearming. Postoperative monitoring and postmortem analysis of animals revealed no evidence of infection, venous thrombosis, or injury related to catheter placement.

There was 1 death before euthanasia and 72-hour scanning in the hypothermia cohort, presumably because of iatrogenic propofol overdose. Hypothermia treatment had ceased 1 day earlier, after which time the baboon had reached normothermia and did not demonstrate any overt residual effects. The animal exhibited normal behavior during the early postoperative period. This baboon was included in the group of animals unable to self-care at 72 hours (see the Outcome section). Because 72-hour or sacrifice infarct volumes and functional assessments could not be obtained, data from this animal did not contribute to imaging and neurological score analysis. In addition, 1 of the animals in the hypothermic cohort had no 72-hour MRI secondary to technical difficulties in the scanning facility. However, this baboon did have 24-hour diffusion-weighted imaging and 10-day T2-weighted MRI.

Physiology
The Table presents physiological variables at baseline, during ischemia, and during early reperfusion for the control and hypothermia cohorts. PCO₂ values were maintained in the range of 35 to 40 mm Hg throughout the study. No differences existed in pH and Po₂ measurements across cohorts (P=NS). MAPB was titrated to 60 to 80 mm Hg by fluid balance, anesthetic management, and a phenylephrine hydro-
chloride drip. Heart rate remained between 70 and 100 bpm (P=NS between cohorts). Hematocrit, white blood cell counts, platelet counts, blood glucose, and serum chemistries were similar for the hypothermic and normothermic cohorts throughout the experiment (P=NS between cohorts). ICP in both hypothermic and normothermic cohorts increased steadily during the perioperative period. The mean postoperative ICP for the hypothermia cohort was not significantly different from that of controls (P=NS; Figure 3A).

**Outcome**

Seventy-five percent of hypothermic animals (6 of 8) were capable of feeding themselves and holding themselves upright at 72 hours, whereas 38% of control animals (3 of 8) were self-caring (P=0.31; Figure 3B). One baboon in the hypothermia cohort (self-sufficient at 72 hours) was deemed unable to feed himself on day 6 and was therefore euthanized at that time (30% 6-day infarct). At 72 hours, the hypothermia cohort (n=6) demonstrated a 17±8% infarct volume, and the control group (n=8) had a mean infarct volume of 32±9% (P=0.12; Figure 4A). At 9 to 10 days, the hypothermia cohort (n=5) exhibited a mean infarct volume of 10±4%, whereas the control group (n=2) had a mean infarct volume of 9±4% (P=0.30). There was a strong correlation between neurological score and infarct volume (r=−0.807, P<0.005) for all animals. At 72 hours, the hypothermia cohort (n=7) had a mean neurological score of 42±9, whereas the control group (n=8) had a mean neurological score of 29±8 (P=0.30; Figure 4B). At 9 to 10 days, the hypothermia cohort (n=5) exhibited a mean neurological score of 58±9, whereas the control group (n=2) had a mean neurological score of 64±32% (P=0.80). Only animals capable of surviving past 72 hours were included in 9 to 10-day infarct volume and neurological outcome analyses. Therefore, the number of baboons (control, n=2; hypothermia, n=5) in this cohort...
Models. 

Successful translation in nonhuman primate stroke raised ethical concerns and emphasize the importance of ischemia that have failed in clinical trials. Poor outcomes have been multiple preliminary reports of the potential utility of cerebroprotective agent in primate stroke. An abundance of hypothermia administration in baboons. This strategy underscores the potential for successful application of hypothermia as a cerebroprotective agent in primate stroke. An abundance of therapies that have proved protective in small animal models of ischemia have failed in clinical trials. Poor outcomes have raised ethical concerns and emphasize the importance of successful translation in nonhuman primate stroke models.

The findings of our study are timely, given the recently published efficacy of mild therapeutic hypothermia in survivors of cardiac arrest. Two separate randomized clinical trials reported improved outcomes in comatose patients after resuscitation from out-of-hospital cardiac arrest. There have been multiple preliminary reports of the potential utility of hypothermia in patients with stroke despite the highly publicized failure of a well-designed multicenter NIH-funded head trauma hypothermia trial. What is clear from the small case series of hypothermia in human stroke and the multicenter head trauma trial is that current surface or nonconvective catheter techniques are probably too slow and/or inconsistent to be efficacious. In addition, the neuroprotective benefits in these studies may have been negated by hypothermia-associated cardiac instability, hypotension, coagulopathy, fever, infection, and cerebral edema. In these studies, the duration of hypothermia was prolonged, usually 48 hours. Despite such complications, several groups continue to advocate the use of prolonged cooling. In the late 1970s, Steen and colleagues treated primates with 48 hours of 29°C hypothermia, which resulted in worsened outcome. Our data suggests that prolonged use of hypothermia may be unnecessary. This study yielded a 50% reduction in 72-hour infarct volume without any complications when hypothermia was maintained for only 6 hours. More definitive nonhuman primate studies may determine, with greater statistical strength, the optimal therapeutic window and treatment duration before large-scale hypothermia trials in human stroke. The catheter described here will likely enhance the efficacy of clinical studies by enabling rapid and precise temperature control and delivery. A brief episode of mild core hypothermia instituted at a clinically relevant time point with the described intravascular cooling technique provides safe, rapid, reproducible hypothermia. The development of convection-enhanced intravascular cooling marks a paradigm shift in hypothermia administration and has implications for the management of tissue injury in a variety of organ systems and settings.

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References

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